Journal of Applied Horticulture, 17(3): 222-224, 2015

Enhancement of *in vitro* corm production in *Gladiolus* by periodically replacement of liquid media using coir matrix

A.K. Bera[1], T.R. Maity[2], A. Samanta[1], A. Dolai[1], B. Saha[2] and S. Datta[2]*

*[1]Mahishadal Raj College, Mahishadal-721 628, West Bengal, India. [2]Haldia Institute of Technology, Haldia-721 657, West Bengal, India. *E-mail: dattasiraj@hithaldia.in*

Abstract

An efficient protocol was developed for mass scale *in vitro* corm production of *Gladiolus* (cv. 'White Friendship') using liquid culture and coconut coir explored as a matrix. The culture was initiated from the basal portion of the innermost leaf of the sprout. The cut surface of responding explant was swelled in MS solid basal media supplemented with 2 mg L^{-1} NAA. Ten to fifteen shoot buds were observed when the responding explant was transferred in MS media with 2 mg L^{-1} BAP and 0.2 mg L^{-1} NAA. The high frequency of *in vitro* corms were initiated in liquid MS media supplemented with 0.5 mg L^{-1} NAA and 6% sucrose in coir matrix. The corms with highest mean fresh weight (4570 mg) and diameter (25 mm) developed on periodically replaced media (every three weeks) than unchanged media. Using RAPD profiling, the genetic fidelity of *in vitro* raised corms were tested.

Key words: Efficient protocol, coconut coir, *in vitro* corms, *Gladiolus,* RAPD, genetic fidelity

Introduction

Gladiolus (hybrid cultivars of *G. hybridus* Hort.; Family: Iridaceae) is one of the valuable cut flowers grown throughout the world for its attractive spikes and wide range of colors. It is commercially propagated through corms and cormlets (Bose *et al*., 2003). But the major constraint in its cultivation is the non-availability of a large quantity of propagules as well as loss of stored corms by *Fusarium* corm rot disease (Memon *et al*., 2014). Thus, the mass propagation through vegetative way was hampered due to the unavailability of insufficient corm quantity (Sajjad *et al*., 2014). *In vitro* techniques were adopted as an alternative to the conventional methods for production of cormlets in different cultivars *viz*., 'Eurovision' (Ziv, 1989), 'Friendship' (Hussain *et al*., 1994; Dantu and Bhojwani, 1995), 'Green Bay' (Sen and Sen, 1995), 'Golden Wave' (Sinha and Roy, 2002), 'Pacifica' (Roy *et al*., 2006), 'Pacific Pink', 'May Queen', 'Sharone' (Thun *et al*., 2008; Memon *et al*., 2010), 'Green Bay', 'Intrepaid' 'Sabnam', 'White Friendship', 'Red Ginger' (Saha *et al*., 2013), 'White Friendship', 'Peter pears' (Memon *et al*., 2014) to overcome this constraint in *Gladiolus* production. However, the *in vitro* cormlet size is still the matter of concern for a commercial success using this technology. The present investigation is designed to establish an *in vitro* protocol for cormlet production with the enhanced cormlet size and fresh weight than previously reported (Saha *et al*., 2013).

Materials and methods

Tissue culture: Healthy corms of *Gladiolus* (cv. 'White Friendship') were collected from local farmers of Panskura, Purba Medinipur, West Bengal, India. The corms were germinated in earthen pots with sand periodically moistened by water (Fig. 1, a-b). The basal portion of the innermost leaf was taken as an explant from the sprouting. The explants were thoroughly washed with Tween 20 (0.1% w/v; Himedia, Mumbai, India) for 15 min and then surface was sterilized by 0.1% $HgCl_2$ (Merck, Mumbai, India) for 7 min. Then the explants were rinsed with sterile distilled water four times for 2-3 min duration. After that these sterilized explants were inoculated on MS media (Murashige and Skoog, 1962) supplemented with 2 mg L^{-1} NAA (α-naphthalene acetic acid, Hi-media) with 3% sucrose. About two to three weeks later the explants started to swell and then these were transferred onto MS media containing 2 mg L^{-1} BAP (6-benzyl amino purine, Hi-media) and 0.2 mg L^{-1} NAA for shoot bud initiation. After three weeks the shoots were transferred to MS media supplemented with 0.2 mg L^{-1} NAA for elongation and maturation of the shoot buds. When shoots get elongated they were transferred to sterile coir (coconut husk) as matrix (Gangopadhyay *et al*., 2002) supplemented with 0.5 mg L^{-1} NAA and 6% sucrose. The increased sucrose concentration is important for corm formation as reported previously (Saha *et al*., 2013). Two different sets of medium for *in vitro* corm inducetion were maintained; in first set the medium was not replaced throughout the experiment and in second set the liquid media were replaced by same fresh media after every three weeks. The initiation of corms started from 2-3 weeks. The diameter and fresh weight was recorded after maturation of the corm. Each set had 30 replicates and the whole experiment was repeated thrice.

RAPD analysis: DNA of young leaf from *in vitro* raised corms were extracted using CTAB method (Rogers and Bendich, 1988). RAPD reaction was performed as per Saha *et al*. (2013) using 10 oligonucleotide primer (OPA1 - OPA10). Amplification of DNA was done using thermal cycler (Applied Biosystem, USA) with an initial denaturation of 2 min at 94 °C, 45 cycles of 1 min at 94 °C (denaturation), 1 min at 35 °C (annealing) and 1 min 30s at 72 °C (extension). After completion of cycles, the PCR mix was hold for 5 min at 72 °C for primer extension. PCR reaction (20 µL) was made up of 1x buffer, 0.2 mM $MgCl_2$, 0.2 µM primer, 100 ng template DNA and 1 µL Taq DNA polymerase (3 units). Thermocycler amplified products were electrophoresed in 1.2% agarose gel with BIOLINE, Hyper Ladder II served as molecular weight marker.

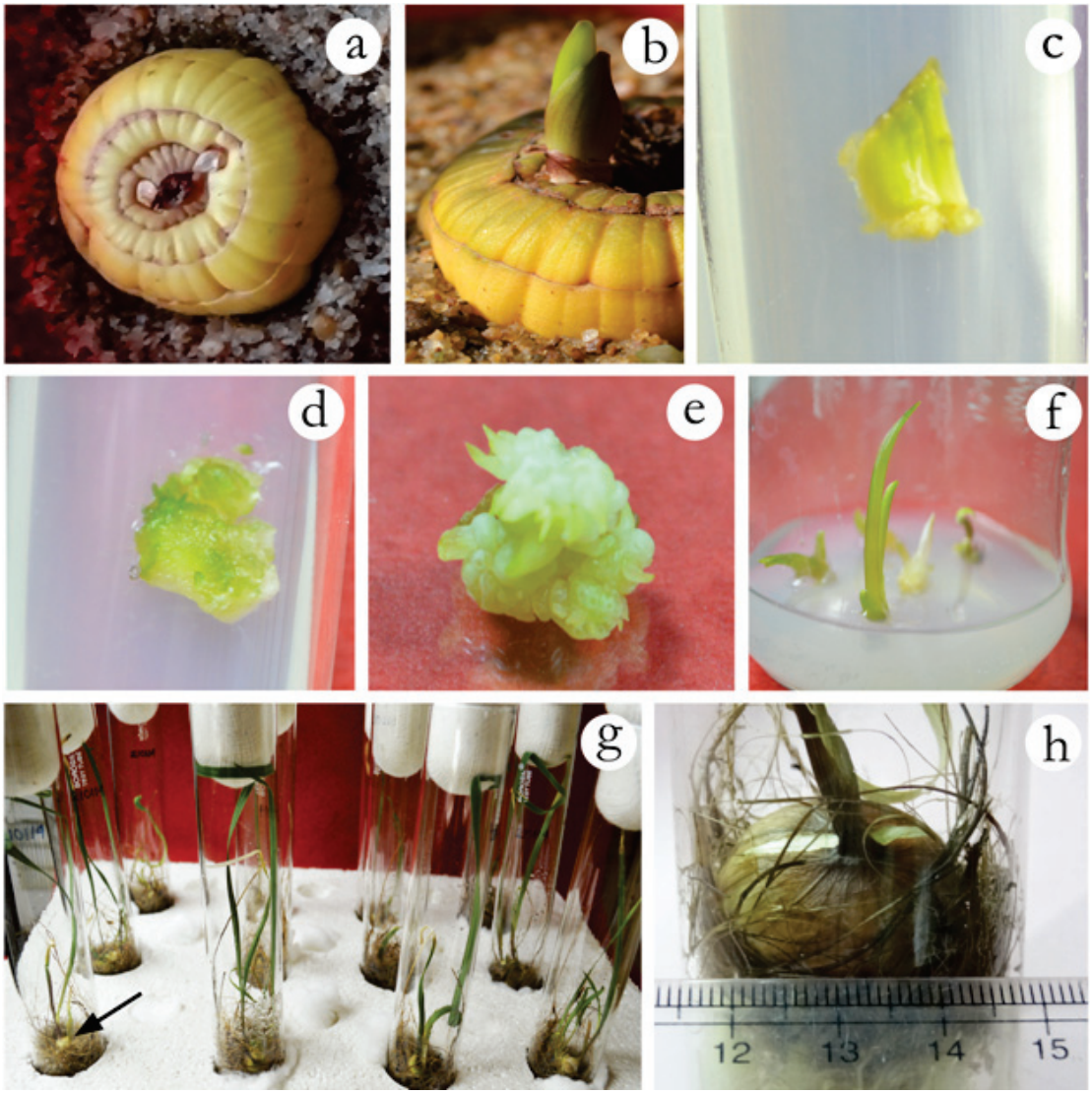

Fig. 1. Flow chart with pictorial representation of different stages of *in vitro* corm formation of *Gladiolus*. (a) mother corm (b) sprouting (c) basal portion of innermost leaf of sprouting in the media (d) swelling of the cut surface (e) multiple shoot cluster (f) elongated shoot (g) mass induction of *in vitro* corms in liquid culture with coir matrix (h) magnified view of an *in vitro* corm.

Statistical analysis: Standard errors of the means were calculated and LSDs were performed to check the level of significance of the difference between the means of fresh weight and diameter of the *in vitro* corms developed in control and replacing media.

Results and discussion

Swelling was observed in the cut surface of (kept on MS medium supplemented with 2 mg L^{-1} NAA) the basal portion of the innermost leaf of newly sprouted *Gladiolus* (Fig. 1, c-d). The basal portions of the innermost leaves were most responsive interns of direct organogenesis, other part of the leaves did not result in uniform response rather it gave rise creamy white callus. The cluster of shoot buds (Fig. 1, e) was visible after three weeks, when the responding explants were transferred in shoot initiation medium (0.2 mg L^{-1} NAA and 2.0 mg L^{-1} BAP). These shoot clusters developed into individual plants when they were placed in MS medium supplemented with 0.5 mg L^{-1} NAA (Fig. 1, f).

Osmoticum in the form of high concentration of sucrose (6%), together with auxin (NAA, 0.5 mg L^{-1}) in liquid medium with coir as a matrix was used to induce *in vitro* corm earlier (Saha *et al*., 2013). Micro corm may also develop in the agar-gelled media but liquid system was much more efficient (Roy *et al*., 2006). Sub culturing was difficult in agar gelled medium due to mechanical injury of the roots reported (Ziv, 1989; Hussain *et al*., 1994; Dantu and Bhojwani, 1995; Sen and Sen, 1995). Present investigation shows that the replacement of media with fresh one after every three weeks produced more healthy and bigger size of corms than unchanged media on coir (Table 1). The stunted growth in unchanged media was probably due to the leaching of polyphenols and other secondary metabolites of the plants which may be toxic to its growth. Changing the older media with new media may remove this toxic stress on corm growth, thus in replacing media the diameter (25 ± 2.15 mm) and fresh weight (4570 ±254 mg) of *in vitro* corms were significantly high ($P \leq 0.05$) as compared to control culture. The steps of *in vitro* corm production (Fig. 1g-h) have been represented in Fig. 2 for effective utilization of this protocol.

Table 1. *In vitro* corm fresh weight and diameter in periodically replaced medium and control

Weeks after corm initiation	Fresh weight (mg)		Corm diameter (mm)	
	Control	In replacing media	Control	In replacing media
1	781±71	788±76	6±0.31	6±0.49
3	1801±102	1811±131	11±0.79	11±0.97
6	2324±110	2513±197	14±0.95	15±1.06
9	-	4234±225	-	19±1.13
12	-	4570±254	-	25±2.15
Mean	1635.3	2783.2	10.3	15.2
LSD ($P \leq 0.05$)	2951.67		14.9	

Assurance of clonal fidelity is another important aspect for establishing an *in vitro* protocol prior to commercialization. Previously PCR-based molecular markers have been used for

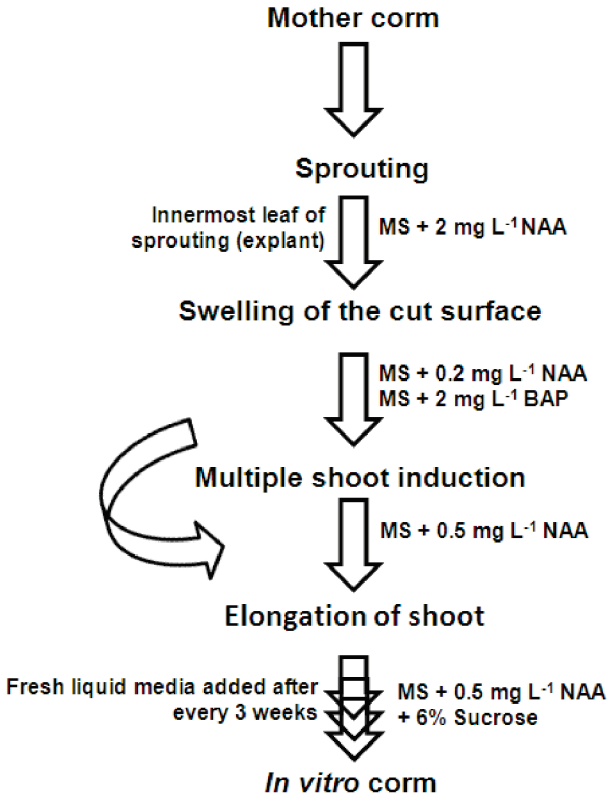

Fig. 2. Flow chart of cultivar-independent protocol for *in vitro* corm production of *Gladiolus*

Gladiolus to assess the clonal fidelity (Roy *et al*., 2006; Saha *et al*., 2013). In the present investigation, RAPD profile of randomly selected samples (sprouted leaf) of mother and *in vitro* corms were identical (Fig. 3), suggesting genetic fidelity.

Upgradation of existing methodology would be valuable for all the commercial practice, *in vitro* corm production of *Gladiolus* not being the exception to that. The present investigation not only improved the existing methodology but also paved the way for a rapid *Gladiolus* hybrid evaluation.

Acknowledgment

The work was supported by the grant aided by Science and Engineering Research Board, Government of India (SR/FT/LS-102/2010).

References

Bose, T.K., L.P. Yadav, P. Pal, P. Das and V.A. Parthasarathy, 2003. *Commercial Flowers*. Volume II, Naya Udyog, Bidhan Sarani, Kolkata.

Dantu, P.K. and S.S. Bhojwani, 1995. *In vitro* corm formation and field evaluation of corm-derived plants of Gladiolus. *Scientia Hort*., 61: 115-129.

Gangopadhyay, G., S. Das, S.K. Mitra, R. Poddar, B.K. Modak and K.K. Mukherjee, 2002. Enhanced rate of multiplication and rooting through the use of coir in aseptic liquid culture media. *Plant Cell, Tissue and Organ Cult*., 68: 301-310.

Hussain, S.C.T., C.K. Geetha, P.K. Rajeevan and P.K. Valsalakumari, 1994. Plant regeneration from root derived callus in Gladiolus. *J. Ornament. Hort*., 2: 46-50.

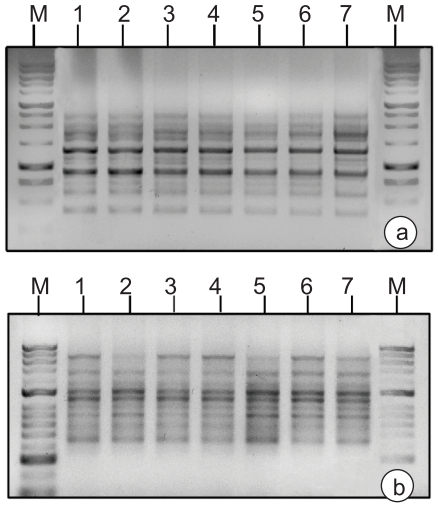

Fig. 3. RAPD profile of randomly selected samples (sprouted leaf) from *in vitro* corms and mother corms. (a) RAPD profile of OPA-3 and (b) OPA-7; M: DNA molecular wt. marker (BIOLINE, Hyper Ladder II); 1: mother plant; 2-7: randomly selected samples from *in vitro* corms.

Memon, N., M. Qasim, M.J. Jaskani and R. Ahmad, 2010. *In vitro* cormel production of Gladiolus. *Pak. J. Agr. Sci*., 47: 115-123.

Memon, N., M.J. Jasakni, M. Qasim and N. Sharif, 2014. Cormel formation in Gladiolus through tissue culture. *Pak. J. Agr. Sci*., 51: 475-482.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with Tobacco tissue culture. *Physiol. Plant.*, 15: 473-497.

Nhut, D.T., J.A.T. da Silva, P.X. Huyen and K.Y. Paek, 2004. The importance of explant source on regeneration and micropropagation of Gladiolus by liquid shake culture. *Scientia Hort.*, 102: 407-414.

Rogers, S.O. and A.J. Bendich, 1988. Extraction of DNA from plant tissues. In: *Plant Molecular Biology Manual*, Schilperoort RA (eds), Kluwer Academic Publishers, The Netherlands. pp. 226-231.

Roy, S.K., G. Gangopadhyay, T. Bandyopadhyay, B.K. Modak, S. Datta and K.K. Mukherjee, 2006. Enhancement of *in vitro* micro corm production in gladiolus using alternative matrix. *Afr. J. Biotechnol*., 5: 1204-1209.

Saha, B., A.K. Datta, S. Datta, and J.A.T. da Silva, 2013. *In vitro* corm development, field evaluation and determination of genetic stability of corm-derived elite Gladiolus germplasm. *Floricult. Ornament. Biotechnol*., 7: 82-85.

Sajjad, Y., M.J. Jaskani, M.Y. Ashraf, M. Qasim and R. Ahmad, 2014. Response of morphological and physiological growth attributes to foliar application of plant growth regulators in Gladiolus 'White Prosperity'. *Pak. J. Agr. Sci*., 51: 123-129.

Sen, J. and S. Sen, 1995. Two-step bud culture technique for a high frequency regeneration of Gladiolus corms. *Sci. Hort*., 64: 133-138.

Sinha, P. and S.K. Roy, 2002. Plant regeneration through *in vitro* cormel formation from callus culture of *Gladiolus primulinus* baker. *Plant Tissue Cult*., 12: 139-145.

Thun, V., D.H. Goo, M.H. Kim, N.S. Byun and K.W. Kim, 2008. Effect of *in vitro* culture environments and culture methods on cormlet formation of Gladiolus. *Hort. Environ. Biotechnol.,* 49: 114-120.

Ziv, M. 1989. Enhanced shoot and cormlet proliferation in liquid cultured Gladiolus buds by growth retardants. *Plant Cell*, *Tissue Organ Cult*., 17: 101-110.

Received: June, 2015; Revised: July, 2015; Accepted: August, 2015